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Review

THE ROLE OF GRAPEVINE LEAF MORPHOANATOMICAL TRAITS IN DETERMINING CAPACITY FOR COPING WITH ABIOTIC STRESSES: A REVIEW

CARACTERISTICAS MORFOANATÓMICAS DA FOLHA PARA A DETERMINAÇÃO DA CAPACIDADE DE ADAPTAÇÃO DA VIDEIRA AOS STRESSES ABIÓTICOS: REVISÃO

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ABSTRACT

Worldwide, there are thousands of *Vitis vinifera* grape cultivars used for wine production, creating a large morphological, anatomical, physiological and molecular diversity that needs to be further characterised and explored, with a focus on their capacity to withstand biotic and abiotic stresses. This knowledge can then be used to select better adapted genotypes in order to help face the challenges of the expected climate changes in the near future. It will also assist grape growers in choosing the most suitable cultivar(s) for each *terroir;* with adaptation to drought and heat stresses being a fundamental characteristic. The leaf blade of grapevines is the most exposed organ to abiotic stresses, therefore its study regarding the tolerance to water and heat stress is becoming particularly important, mainly in Mediterranean viticulture. This review focuses on grapevine leaf morphoanatomy - leaf blade form, leaf epidermis characteristics (cuticle, indumentum, pavement cells and stomata) and anatomy of mesophyll - and their adaptation to abiotic stresses. *V. vinifera* xylem architecture and its adaptation capacity when the grapevine is subjected to water stress is also highlighted since grapevines have been observed to exhibit a large variability in responses to water availability. The hydraulic properties of the petiole, shoot and trunk are also reviewed. Summarising, this paper reviews recent advances related to the adaptation of grapevine leaf morphoanatomical features and hydraulic architecture to abiotic stresses, mainly water and heat stress, induced primarily by an ever-changing global climate.

RESUMO

Em todo o mundo existem milhares de cultivares de *Vitis vinifera* para produção de vinho representando uma enorme diversidade morfológica, anatómica, fisiológica e molecular mas que necessita de ser mais caracterizada e explorada, com foco nas suas capacidades para suportar stresses bióticos e abióticos. Esse conhecimento poderá então ser usado para selecionar genótipos adaptados, a fim de ajudar a enfrentar os desafios inerentes às mudanças climáticas. Também ajudará os viticultores na escolha da(s) cultivar(es) mais adequada(s) para cada *terroir*, sendo a adaptação à seca e ao calor uma característica fundamental. A folha é o órgão mais exposto aos stresses abióticos pelo que o seu estudo relativamente à tolerância ao stress hídrico e térmico assume grande importância, particularmente na Viticultura mediterrânica. Esta revisão foca-se, por isso, na morfoanatomia da folha - forma do limbo, características da epiderme (cutícula, indumento, células epidérmicas propriamente ditas e estomas) e anatomia do mesófilo - e sua adaptação aos stresses abióticos. Também se revê a arquitetura do xilema da videira, caule e pecíolo, e as suas propriedades hidráulicas, visto ser observável grande variação na forma como as videiras respondem à disponibilidade hídrica. Resumindo, esta revisão sintetiza os resultados mais recentes relacionados com a adaptação das características morfoanatómicas da folha e da arquitetura hidráulica da videira aos stresses abióticos, principalmente hídrico e térmico, induzidos principalmente por um clima global em constante mudança.

Keywords: hydraulic conductivity, leaf epidermis, mesophyll, morphoanatomy, stomata, xylem.

Palavras-chave: condutividade hidráulica, epiderme foliar, estomas, mesófilo, morfoanatomia, xilema.

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INTRODUCTION

The grapevine (*Vitis vinifera* L.) is amongst the most economically important fruit crops globally, with representation in more than 90 countries (FAO-OIV, 2016).Vineyards are largely found in drought prone areas, although grape growers are increasingly finding ways of producing wine successfully in colder countries such as England and Sweden as well as tropical climates, such as in Thailand and Brazil.

In many Mediterranean countries, the wine industry is of great socioeconomic importance, contributing to economic stability and development (Fraga *et al*., 2016). A better understanding of climate change will assist in vineyard adaptation in order to guarantee fruitful future harvests. Grapevine thermal requirements are increasingly demonstrating that climate change is contributing to the review of the distribution of cultivars as well as the displacement of some due to an inadequate capacity to cope with seasonal temperature changes; also highlighting the importance of cultivar-dependent analyses (Santos *et al.,* 2018). Historically, the grapevine has been considered as a drought tolerant species (Schultz, 2003; Schultz and Stoll, 2010), which is well adapted to the Mediterranean climate and has the ability to alter growth and physiology in order to store current reserves, and to control future demand for resources (Schultz, 2003). However, the increasingly variable climatic conditions, such as long periods of heat and dry weather (Dinis *et al.,* 2014), can considerably affect the canopy growth, productivity and berry composition (Chaves and Rodrigues, 1987; Escalona *et al*., 1999; Flexas *et al*., 2002, 2010; Chaves *et al*., 2007, 2010). Climate change also contributes to more extreme weather events, which can occur on a sporadic basis, such as the heat wave experienced in Portugal in August 2018. The pressure induced on water resources is an increasingly vicious cycle as the demand for irrigation soars and further highlights the need to understand how grapevines can sustain yields while improving water use efficiency (Chaves *et al*., 2010; Mosedale *et al.*, 2016).

It is widely recognised that plants have developed features and functions to assist them in unfavourable growing environments. Leaves in particular are known to have various adaptive mechanisms to help withstand abiotic pressure, in particular, drought and heat stress (Rizhsky *et al.,* 2004; Mittler, 2006; Kotak *et al.,* 2007) as well as light stress (Chavarria *et al*., 2012). Despite these known generalisations between abiotic factors and the reaction of plants, there are few studies that look at leaf micromorphology traits as a way of actually explaining heat and water stress tolerance (Gómez-del-Campo *et al.,* 2003; Koundouras *et al*., 2008; Costa *et al.,* 2012). Such studies may lead us to label some cultivars as being more drought tolerant than others, due to the way they have developed leaf features to support a positive coping capacity when confronted with climate changes. When addressing a plant's mechanism for high temperature survival, long-term phenological and morphological adaptations can be assessed as well as short term avoidance or acclimation mechanisms (Wahid *et al.,* 2007). Photosynthesis is one of the physiological processes which is most sensitive to temperature, as it has a direct effect on enzyme activity and an indirect effect in stomatal opening. The optimum temperature in grapevines for photosynthetic activity is from 20 to 35 °C (Chaves *et al*., 2016). This fairly wide temperature range is due to the large intra-species variability of grapevines and their capacity to adapt to the growing environment.

But the high degree of heterogeneity of this species is also one of the reasons for the differences seen in response to abiotic stress; such as drought, triggering stomatal response and differences in water use efficiency (Chaves and Rodrigues, 1987, 2010; Bota *et al*., 2001; Medrano *et al*., 2003).

In addressing and identifying the key morphological and anatomical grapevine traits that respond to drought and extreme heat, researchers can identify and develop the necessary phenotyping required in the search for more drought-tolerant cultivars. Drought tolerance in this context can be linked with water use efficiency, leaf dehydration tolerance, plant hydraulic conductance, stomatal conductance, rooting depth and embolism repair (Hopper *et al*., 2014). Furthermore, a greater understanding of grapevine coping capacity could help with the conservation of cultivars as well as assisting with rootstock and clonal selection during the process of genetic breeding.

This review summarises the main findings of how grapevine leaf and morphoanatomical traits, as well as the petiole and stem xylem relationship with hydraulic conductivity, contribute to the plant's coping capacity when confronted with climate change, both in red and white cultivars.

GRAPEVINE MORPHOANATOMY:

ADAPTATIONS TO ABIOTIC STRESS

Differences have been observed between *V. vinifera* cultivars by studying the leaf morphoanatomical traits. This chapter and sub-sections will explore the leaf morphoanatomical traits found in grapevines, focusing on features including the morphology and anatomy of the leaf blade. In particular, leaf shape and area, characteristics of the epidermis - indumentum, cuticle, stomata and epidermis cells – and anatomy of the mesophyll. These traits will be

reviewed by paying attention to their degree of adaptation to abiotic factors.

Leaf Shape and Area

Leaf shape presents a large diversity among *V. vinifera* cultivars, contributing to various functions, such as thermoregulation, hydraulic constraints and light interception through the canopy (Chitwood *et al.*, 2016). Changes in leaf shape can be environmentally induced, demonstrating that leaves are capable of responding to the surrounding climate conditions in a flexible manner (Royer *et al.*, 2009). Genetically, leaf shape is considered heritable and regulated by *loci* that are almost totally unrecognisable at molecular level (Langlade *et al*., 2005; Tian *et al.*, 2011; Chitwood *et al.*, 2013, 2014).

Grapevine leaf area can be correlated with the rate of transpiration. However, grapevine leaf area varies with the cultivar (Chitwood *et al.*, 2014), the ecosystem ecology (Gürsöz, 1993), the phase of shoot development, and the leaf position along the shoot (Lopes and Pinto, 2005), and also with cultural practices such as the pruning system, fertilisation (Keller and Koblet, 1995), irrigation (Lopes *et al.*, 2011) and canopy management (Smart and Robinson, 1991; Lopes *et al*., 2020), among others.

On a macro-morphological scale, significant variation in leaf size has been noted throughout the canopy (Bodor *et al*., 2018), with the basal and apical leaves being smaller than those found in the middle of the shoots. In a recent study, it has been suggested that the variability in leaf size along the shoot is possibly caused by heteroblasty and ontogeny (Bodor *et al.*, 2019). This study also presents evidence of patterns in leaf blade thickness. Leaves from the lateral shoots tend to be thinner and smaller than those from primary shoots. This size differentiation could be explained by ontogeny; however ecological conditions and abiotic stresses can also affect the rate of emergence and growth of new leaves (Bodor *et al*., 2019).

Jones (2014) notes that smaller leaves have a better control over leaf temperature due to their higher boundary layer conductance. Gago *et al.* (2019) found that 'Grenache noir' leaves have a smaller total surface area than 'Syrah' leaves, which has been considered as a cultivar specific adaptation correlated with drought tolerance (Doupis *et al*., 2016). Under heat stress conditions, some cultivars exhibit a smaller total leaf area and thicker leaf blades, which also means a thicker mesophyll (Salem-Fnayou *et al.*, 2011). Grapevines are also able to adjust the leaf area to the degree of water availability by promoting leaf senescence as a drought stress response (Jones, 2014, Chaves *et al*., 2007; Hochberg *et al.*, 2017).

Characteristics of the Leaf Epidermis

Cuticle

The primary function of the plant cuticle is as a barrier to water permeability, preventing the evaporation of water from foliar tissues. The cuticle, which is present on both epidermal surfaces, is the outermost layer of the cell wall, thus being the part of the plant which is most exposed to the surrounding environment. It is a lipophilic layer made up of both cutin and epicuticular waxes (Dhanyalakshmi *et al*., 2019). As a consequence of its exposure, the cuticle acts as a physical barrier, aiming to protect the plant from both abiotic and biotic factors, thus protecting the plant from possible desiccation. Research also shows that the cuticle plays an active role in providing resistance against various pathogens (Ziv *et al*., 2018). In this light, the lipophilic layer has been described as a 'mechanical obstacle' due to its function as a barrier to work against the entry of fungi and pathogenic bacteria (Mendgen, 1996). Cuticular waxes form a hydrophobic layer, capable of regulating gas exchange and non-stomatal water loss as well as the stresses outlined above (Dhanyalakshmi *et al*., 2019). Epicuticular waxes have also been cited as a barrier against water loss by evaporation and protection against the loss of inorganic and organic compounds by the act of leaching from the interior of the plant tissues (Riederer and Markstädter, 1996). The influence that the waxy layer has on the path of any vapours, will not only affect the quantity of water lost, but also affect the waterproofing capacity of the epidermis (Martin and Juniper, 1970). It has been understood that studying the cuticles of plants can provide us with information surrounding the plant's response to abiotic stress as well as the plant's habitat (Martin and Juniper, 1970). The effectiveness of the cuticle under stress conditions depends on its thickness as well as the wax properties, which are known to differ between plant species (Shepherd and Griffiths, 2006). Consequently, it is commonly understood that the thicker the cuticle, the lower the rate of cuticular conductance and, as a result, less water is lost (Medri and Lleras, 1980). As a way of acting as a barrier to abiotic stress, environmental stresses such as drought, change the form of the plant cuticle. In this instance, the cuticle has been observed as increasing in stiffness and quality (Domínguez *et al.*, 2011), most probably also due to the fact that the cuticle is a 'flexible biopolymer' with rheological characteristics, thus helping it to adapt dynamically to climate events (Edelmann *et al*., 2005).

Two studies in Portugal that investigated the morphoanatomy of the cultivars 'Aragonez', 'Cabernet Sauvignon', 'Syrah' and 'Touriga Nacional' (Monteiro *et al*., 2013) and 'Alvarinho', 'Arinto', 'Encruzado', 'Macabeu', 'Moscatel Galego', 'Moscatel de Setúbal' and 'Viosinho' (Teixeira *et al*., 2018) concluded that the thickness of the upper cuticle showed little variation and tended to be thin, with cuticular striations observed

on both the upper and lower surfaces. These striations were occasionally more visible around the stomata (Figure 1A). In the Teixeira *et al.* (2018) study, a higher cuticle thickness was noted in the cultivars, 'Viosinho' and 'Macabeu', as compared with other white cultivars referred above, both of which originate from hot and dry Mediterranean regions. It has been suggested that cuticular thickness in grapevine leaves could be correlated with the growth conditions, in particular drought and heat stress, as well as the genotype. When subjected to heat stress conditions, a fairly significant decrease in the thickness of the cuticle has been detected with leaves showing a 'folded' cuticle and pectocellulosic wall on the upper epidermis (Salem-Fnayou *et al.,* 2011). The lower cuticular conductance induced by a thicker cuticle, means that there was a lower rate of water loss despite the other cultivars in the study under the same atmospheric demand. These results indicate that these characteristics translate to a likely adaptation of these cultivars to the climatic conditions of their geographical origin (Jones, 2014). A comparison of six genotypes of *Vitis* spp. (five being *V. vinifera* and the other *V. riparia* Michx) revealed that the cuticle of the abaxial side tended to be thinner than that of the adaxial side, this being a common feature of many other dicots (Boso *et al*., 2010).

Leaf Indumentum

The grapevine leaf indumentum has long been an ampelographic descriptor, used for describing and classifying grapevine cultivars (Galet, 2000; Cabello *et al*., 2011; Atak *et al*., 2014); one of the reasons being that it is one of the most stable traits among grapevine cultivars, showing little change under shifting environmental conditions (Viala and Vermorel, 1901). The leaf indumentum exhibits a complex structure and appearance that makes any sort of quantification difficult (Monteiro *et al*., 2013). However, it is known that the high trichome density displayed on grapevine leaves, is an important contributor to leaf radiation absorptance, hence modulating carbon, water and the energy balance of the leaf (Mershon *et al*., 2015). Trichome density is assessed visually by looking at the spatial density of growth on the abaxial leaf surface (Gago *et al*., 2016).

Although leaf trichomes may contribute to drought resistance, until now this trait does not determine how grape growers select cultivars to plant, as the dispersal of autochthonous cultivars witnessed nowadays, is generally in line with other more desirable and visible traits (Gago *et al*., 2016).

Despite the differences observed between cultivars, one common feature is that only the surface of the lower epidermis is pubescent (Boso *et al*., 2011; Monteiro *et al*., 2013; Teixeira *et al*., 2018). When observed under a microscope, authors have used different descriptors to report their observations. Early investigations by Pratt (1974) described the vine trichome as being dead and woolly or living and woolly, uni- or pluricellular, twisted or cylindrical and flat. The difference in form among prostrate trichomes, being cylindrical or flat, could be explained by age. The cylindrical form is more abundant in young leaves and the flat form tends to be more common in mature leaves (Boso *et al*., 2011). This aging factor probably explains different fluorescence properties observed in both types of prostrate trichomes (Gago *et al.,* 2016). Prostrate trichomes are easily removed from the leaf surface and a length of around 2000 μm (approximately 10 times longer than erect trichomes) has been observed, with the transverse cell walls being thinner than the outer walls (Gago *et al.,* 2016).

On mature leaves two different types of trichome were classified and described as: i) very long hairs unicellular and normally flat with a helicoidal rolling (Monteiro *et al*., 2013), ii) uni- and pluricellular uniseriate and non-glandular hairs, which tend to be slightly curved or erect (Figure 1B). It is interesting to mention that the two white Portuguese cultivars, 'Viosinho' and 'Moscatel de Setúbal' only exhibit the multicellular, small, erect or slightly curved hairs on the lower epidermis and no long hairs, indicating that the type of plant hair found in *V. vinifera* can be cultivar specific (Teixeira *et al.,* 2018).

The form of the reclining and erect hairs has been investigated as a way of trying to better understand this part of the leaf's indumentum which has still not been well characterised. Whilst the erect hairs appeared to be small spikes under different magnifications, the reclining hairs take the form of different sized filaments. Boso *et al.* (2011) observed that the cultivar, 'Mencía', did not seem to present any erect or reclining hairs, whilst other genotypes possessed one or the other or both types. This lack of hairs in some cultivars could present disadvantages when choosing appropriate cultivars in light of tolerance to abiotic stresses.

When both erect and prostrate types are present, the erect trichomes act as a support for the entangled prostrate hair types, thus, forming a leaf coating, provided by layers of different kinds of trichomes, particularly visible on transverse sections of adult leaves (Figure 1C).

The leaf hairs of the lower epidermis may also have implications on the epidermal temperature (Karabourniotis *et al*., 1995). In terms of temperature regulation and abiotic stress protection however, pubescence present as trichome layers, was considered a 'light screen' with the ability to protect the leaves from excessive light, particularly if the leaves are young and at a stage in which their photosystems are evolving, resulting in a less

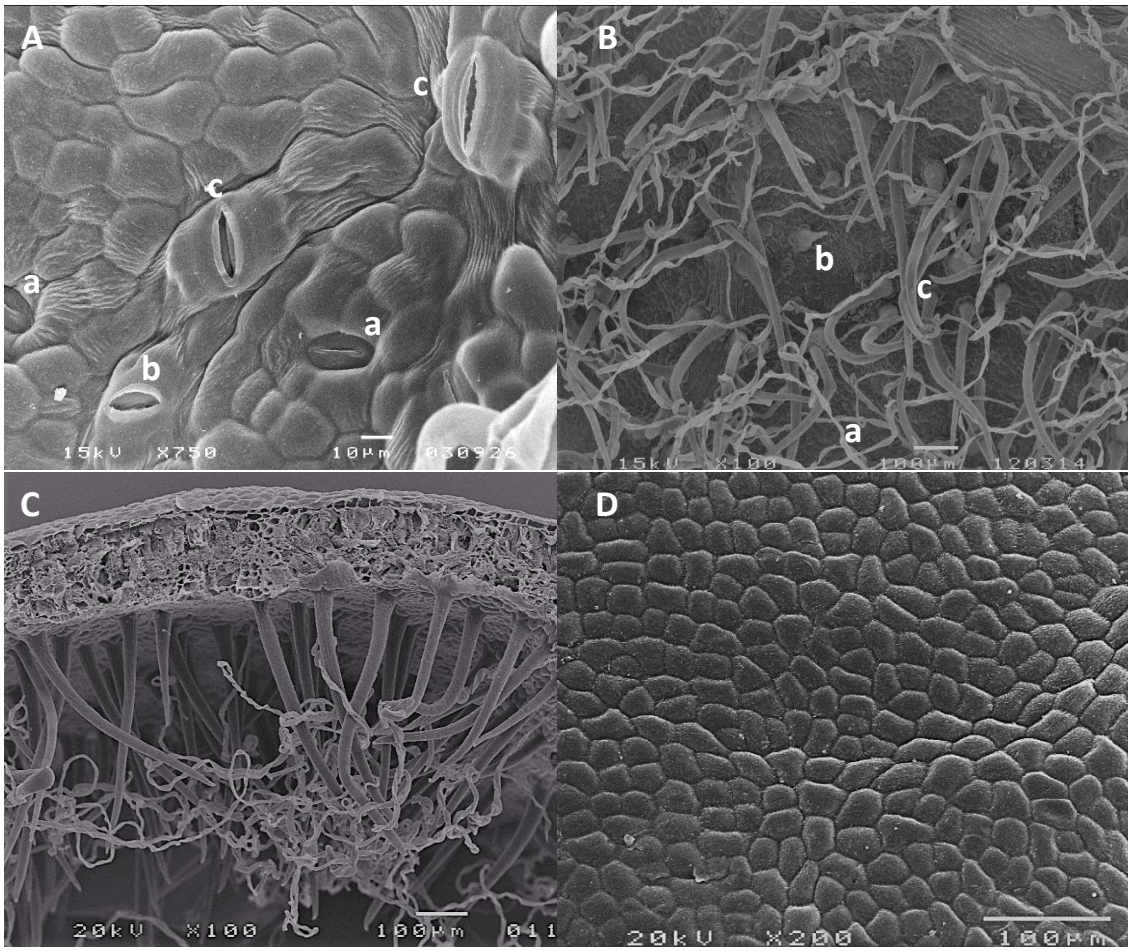

Figure 1. Leaves of different cultivars of *Vitis vinifera* under scanning electronic microscopy: **A -** Cultivar 'Syrah', leaf lower epidermal cells with different shapes and cuticular striations, more visible around the several types of stomata: a – sunken, **b -** same level, **c** – raised; **B -** Cultivar 'Touriga Nacional', leaf lower epidermis with different types of trichome: **a -** long with helicoidal rolling, **b -** short unicellular, **c -** long uni- or pluricellular; **C -** Cultivar 'Touriga Nacional', leaf cross section with layers of different kinds of trichomes; **D -** Cultivar 'Moscatel Galego', leaf upper epidermis with polygonal to round cells.

developed photosynthetic capacity (Liakopoulos *et al*., 2006).

The positioning of the stomata amongst the trichome has been cited as a typical characteristic of plants that are naturally found in dry conditions like xeromorphic plants (Fahn, 1986; Skelton *et al*., 2012). As a result, it may be concluded that cultivars possessing this organisation of the trichome could be better adapted to dry climates. The role of the indumentum in terms of physiological and ecological adaptation is already well known as well as its influence on plant organ development (Theobald *et al*., 1979). Furthermore, Gago *et al.*, (2016) stated that this characteristic can have a useful role in modulating evapotranspiration in grapevine leaves by limiting the air movement around the stomatal pores.

The reflection of a fraction of the incident solar radiation induced by the plant leaf hairs, permits the regulation of the epidermal temperature (Karabourniotis *et al*., 1995) as previously explained, as well as reducing water loss (Levin, 1973; Wagner *et al*., 2004), all of which are important characteristics when considering the vine's resilience to climate change. However, the values of the spectra of absorption, reflection and transmission found in leaves also vary with the thickness, structure, water content, surface morphology and the leaf orientation within the canopy. Reflection can be higher in white and/or pubescent leaves, leaves with high levels of epicuticular wax or leaves with low water content (Jones, 2014).

Upper and lower pavement cells of the epidermis

The pavement cells of the upper and lower epidermis, tightly packed together, serve to prevent excess water loss and to protect other more specialised cells located beneath. Boso *et al.* (2011)

observed that all upper epidermis cells in the genotypes studied are convex and polygonal to round in shape (e.g. Figure 1D), whilst lower epidermis cells displayed greater variability with three types observed: rounded polygonal cells, elongated polygonal cells and sinuous cells (Figure 1A). The same features were also reported for other genotypes and *terroirs* (Monteiro *et al*., 2013; Teixeira *et al.,* 2018). The cultivars studied by these authors displayed upper and lower unistratified epidermal cells with thin walls and a thin cuticle. Transverse epidermal cell surface values have been correlated with ecological conditions, whilst cuticular thickness has been linked to genotype and growth conditions – in particular, drought and heat stress. A larger cellular lumen may be linked to water storage, thus making us ponder its possible physiological significance (Esau, 1977; Dickison, 2000). The grapevine cultivars 'Razegui' and 'Muscat Italia', studied under heat stress, displayed elongated convex epidermal cells with a slightly less sinuous shape than the leaves that were not subjected to heat stress (Salem-Fnayou *et al.,* 2011). Epidermal cells of a particularly large size in the leaf blade samples of the cultivar, 'Athiri' were observed, whilst the 'Syrah' samples showed the smallest forms of epidermal cells (Patakas *et al*., 2003). Therefore it seems that transverse epidermal cell surface area values can differ but these differences are not limited to the geographical origin of the cultivar, nor the degree of abiotic stress.

Stomata

In terms of stomata location, several authors (Pratt, 1974; Bernard, 1978; Duering, 1980; Boso *et al.*, 2011; Monteiro *et al*., 2013; Teixeira *et al.,* 2018) agree that grapevine stomata are mainly found on the lower epidermis. Stomata may be raised above, at the same level or sunken, relative to the surface cells of the lower epidermis (e.g. Figure 1A) (Pratt, 1974). The stomata are surrounded by two guard cells, described commonly as kidney shaped and have a 'substomatal cavity' beneath them (Boso *et al.,* 2010). When the stomata are raised above, each stoma is bordered by subsidiary cells that tend to be curved and the guard cells are also positioned above. Stomata are described as being at the same level when the guard cells are flattened along with the secondary cells and they are sunken when the guard cells are submerged amid the subsidiary cells (Teixeira *et al.,* 2018). The three types of stomata were found in all the aforementioned cultivars studied by Monteiro *et al*. (2013) and Teixeira *et al.* (2018), highlighting that these traits are not considered as wholly cultivar specific but, instead, as adaptations to maintain transpiration at a minimal rate, with sunken and small stomata the most adapted to this trait (Jones, 2014). Indeed, it was found that the raised above stomata presented the largest dimensions, whilst the sunken stomata were always the smallest in terms of width and length (Teixeira *et al*., 2018). Amongst all three types of stomata, the width seemed to be the characteristic that presented the most significant difference between cultivars, although this may vary with leaf hydration.

Stomata density (stomata per mm^2) can differ in function of the stage of development of the grapevine and the environmental conditions under which it is grown (Yan *et al.,* 2017). The stomata density of grapevine leaves can present a large variation. A range of 50 to 400 stomata per mm^2 was reported by Keller (2010), while Monteiro *et al.,* (2013) observed a range of 207 to 286 stomata per mm^2 and Teixeira *et al.,* (2018), 170 to 250 stomata per mm^2. Amongst the cultivars 'Grenache Noir' and 'Syrah', significant differences in stomatal density with pot grown grapevines have also been noted, showing greater differences than field grown plants (Gerzon *et al.,* 2015).

The most common type of stomata observed also vary between cultivars and studies. In the aforementioned study of Teixeira *et al.,* (2018), the most common stomata of seven white cultivars was the 'same level' type. On the other hand, a higher fraction of 'raised above' stomata were observed in the cultivars 'Aragonez' and 'Cabernet Sauvignon', when compared with 'Syrah' and 'Touriga Nacional', which showed a higher percentage of 'same level' stomata (Monteiro *et al.,* 2013).

High stomatal density coupled with lower stomata dimensions are features that minimise transpiration and are considered adaptations of the cultivars to water stress (Jones, 2014; Serra *et al.,* 2017). However, the genotypes with the largest stomata density were those with no reclining hairs, no erect hairs or no hairs at all, whilst genotypes that exhibit few stomata, possess both types of plant hairs (Boso *et al*., 2011).

In heat stress conditions little difference in stomata densities has been noted. Stomata density is thought to be largely conditioned genetically but may also be affected by the environment. Consequently, any variation in density is thought to be an evolutionary adaptation rather than a short-term avoidance mechanism (Salem-Fnayou *et al*., 2011). Stomata density of 'Syrah' is largely unaffected by temperature but the length and width of stomata tended to increase with heat (Sadras *et al.,* 2012). As a result, it may be thought that the longer and wider stomata provided greater plasticity of stomatal conductance under increased temperatures. As the stomata are regulators of gas exchange and control vine water use efficiency, it is also thought that their density could be more affected by drought than by heat stress (Salem-Fnayou *et al.,* 2011). A study under field conditions in the Alentejo wine

growing region of Portugal, to compare the stomatal response to water deficit in the cultivars, 'Aragonez', 'Cabernet Sauvignon', 'Syrah', 'Touriga Nacional' and 'Trincadeira', did not show any correlation between the number of stomata and stomatal conductance (Costa *et al*., 2012). For example, although 'Cabernet Sauvignon' exhibited a high number of stomata, it did not display equally high values of stomatal conductance. This underlines that factors other than stomatal density might contribute to stomatal conductance values (Costa *et al*., 2012). Haworth *et al*. (2021) referred that it is also important to consider the interaction between stomatal morphology and physiological behaviour as a way of interpreting stomatal conductance values.

Mesophyll

Grapevines have dorsiventral leaves, typical of a dicot plant, with an asymmetric mesophyll in which the palisade parenchyma is close to the upper epidermis and the spongy parenchyma is near to the lower epidermis. Even so, two clear variations have been observed in the organisation of the spongy parenchyma (Patakas *et al*., 2003; Boso *et al*., 2010; Salem-Fnayou *et al*., 2011; Monteiro *et al*., 2013; Teixeira *et al*., 2018); the most common being with some intercellular spaces between cells, or on the contrary, the spongy cells are compact with no spaces between them (e.g. Figure 2A and 2B). The palisade layer is made up of one or two layers of column shaped cells which are dotted with 'delineated elliptic' chloroplasts (Salem-Fnayou *et al*., 2011; Monteiro *et al.*, 2013; Teixeira *et al.*, 2018). However, in the cultivars 'Grenache Noir' and 'Syrah', the palisade layer is made up of only one layer of cells (Gago *et al*., 2019). These differences reinforce that grapevine mesophyll thickness and organization generally do not follow a pattern and tend to vary between cultivars in many studies.

In terms of the extent of the palisade and spongy layer of *V. vinifera* palisade layer is approximately 40% of the thickness value observed for the spongy mesophyll layer (Boso *et al*., 2010). However, the palisade mesophyll cells were significantly thicker in the two clones of the 'Albariño' cultivar and about four times thicker than those seen in 'Tempranillo', 'Pinot Noir', 'Cabernet Sauvignon' and 'Touriga Nacional'. These 'Abariño' clones had more elongated palisade cells, with plentiful chloroplasts. Similar results were observed in the cultivars: 'Alvarinho', 'Arinto', 'Encruzado', 'Macabeu', 'Moscatel Galego', Moscatel de Setúbal' and 'Viosinho'. The palisade layer tended to occupy around 40% of the total mesophyll, and the spongy tissue, around 60%, but the palisade tissue of 'Viosinho', occupied around 67% of the total mesophyll (Teixeira *et al*., 2018). Further similarities were noted in the cultivars: 'Aragonez', 'Cabernet Sauvignon', 'Syrah' and 'Touriga Nacional', with the palisade tissue ranging from 35% to 39.5% of the total mesophyll (Monteiro *et al*., 2013). A thicker palisade tissue is thought to help in the assimilation of carbon dioxide, by increasing the number of locations per unit area of the leaf surface for this assimilation (Ennajeh *et al*., 2010). Furthermore, if the stomata are prompted to close in order to conserve water, a thicker palisade layer may aid in maintaining the rate of photosynthetic assimilation (Gago *et al*., 2019).

In addition to phenolic compounds, mucilage and calcium oxalate crystals (raphide type) may also be observed. Plant mucilage are complex polysaccharide polymers usually associated with some proteins. Due to their chemical variability, mucilage might have several functions in plants, differing with the plant species, organ and tissue in which they are accumulated (Taiz and Zeiger, 2004). Its water retention ability has always been known, allowing the storage of huge water reserves in the cells, which helps with drought tolerance (Gago *et al*., 2019). When grapevines are subjected to conditions of water stress, the number of cells containing calcium oxalate and mucilage (idioblasts) tended to increase (Doupis *et al*., 2016) and can be released from the leaf mesophyll when required (Gago *et al*., 2019). The presence of calcium oxalate idioblasts in the mesophyll cells of cultivars (e.g. Figure 2C) has been mentioned (Teixeira *et al.*, 2018), but the search for mucilaginous idioblasts was not included in the study.

GRAPEVINE XYLEM AND WATER TRANSPORT UNDER STRESS

The studies of water transport in plants enable to improve water use efficiency (Jones, 1990) and to evaluate plant's tolerance to drought and habitat adaptability (Sperry and Tyree, 1990; Cochard *et al.*, 1994; Pockman *et al*., 1995). In the light of an increasing interest in global climate change and its implications for grapevine cultivation, a better understanding of xylem histology is fundamental for the identification of hydraulic efficiency and resistance to water stress.

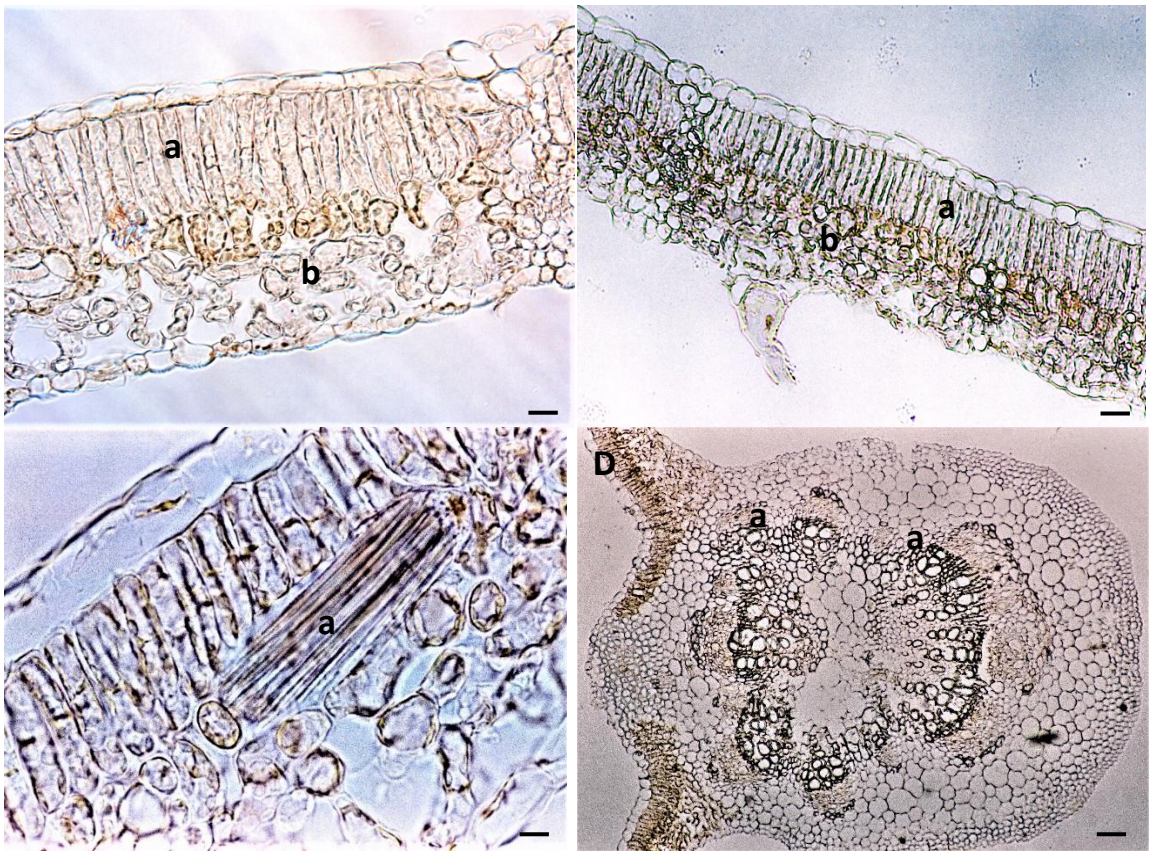

Figure 2. Leaves of different cultivars of *Vitis vinifera* under light microscopy: **A** - Cultivar 'Arinto', leaf cross section: **a** – one or two palisade layers, **b** - spongy parenchyma with many intercellular spaces (x400), scale bar 25 µm; **B** - Cultivar 'Touriga Nacional', leaf cross section: **a** – one or two palisade layers, **b** - spongy parenchyma almost compact (x40), scale bar 25 µm; **C** - Cultivar 'Alvarinho', leaf cross section: **a** - idioblast with raphids needle calcium oxalate crystals (x400), scale bar 18 µm; **D** - Cultivar 'Cabernet Sauvignon', leaf cross section, main vein: **a** - bi-collateral xylem vessels (x40), scale bar 0.04 cm.

Xylem vessels in plants are responsible for water transport from the soil to the leaves, replacing water lost through transpiration. This water transport happens under negative tension and in a metastable state (Quintana-Pulido, 2018).

V. vinifera cultivars have long and wide xylem vessels compared with other woody plants (Brodersen *et al*., 2013b). It is suggested that xylem organization in grapevines could be related to the age or location within the plant, with lateral hydraulic conductivity being common in older grapevine wood, existing mainly through perforated ray cells (Chalk and Chattaway, 1933; Wheeler and LaPasha, 1994; Merev *et al*., 2005; Brodersen *et al*., 2013b;). The xylem properties of mature vine stems have been evaluated and related to water availability during the vineyard establishment period (Munitz *et al*., 2018). The authors conclude that a higher water availability during the first seasons of vineyard establishment, has contributed to the formation of wider xylem vessels and, hence, an increase in hydraulic conductivity. This study was based on the presumption that xylem vessel length and diameter is correlated with the diameter of the stem, meaning that grapevine trunks tend to have wider and longer xylem vessels, compared with young stems (Ewers and Fisher, 1989; Jacobsen and Pratt, 2012; Jacobsen *et al*., 2015). Stem xylem properties vary greatly among grapevines (Schultz, 2003; Quintana-Pulido *et al*., 2018), implying that there will also be intricate differences in hydraulic conductivity between cultivars.

The xylem architecture of *V. vinifera* cultivars is related to hydraulic vulnerability (Choat *et al*., 2010) due to the length and width of xylem vessels. This level of vulnerability varies between cultivars depending on how the cultivar responds to water availability (Chaves *et al*., 2010). Grapevines are generally characterised as a species that can withstand water stress, but research suggests that

they are still particularly susceptible (Choat *et al*., 2010) to drought-induced xylem cavitation when the stress is severe. This susceptibility is also increased with the existence of wide, long xylem vessels because, despite giving way to higher hydraulic conductivity, they are more prone to cavitation (Hargrave *et al*., 1994; Hochberg *et al*., 2014).

Xylem vessels are exposed to biotic and abiotic stresses that can in turn, threaten their function. If the xylem vessels are exposed to drought or freezing for example, a common response would be cavitation, thus causing a decrease in water transport capacity (Pouzoulet *et al.,* 2014). Cavitation induced by water stress is described as a principal drawback in hot and dry climates (McDowell *et al*., 2008; Kursar *et al*., 2009). Furthermore, water stress conditions, also induce a reduction in size of both the diameter and cross-sectional area of the xylem vessels (Lovisolo and Schubert, 1998).

Petiole hydraulic conductivity

The petiole's xylem vessel architecture exhibit a large variability (Schultz, 2003) determining cultivar differences in hydraulic conductivity, transpiration and susceptibility to embolism (Bota *et al.,* 2001; Schultz, 2003; Alsina *et al*., 2007). Petioles have also been found to be very susceptible to embolism, when compared with stems and roots (Lovisolo *et al*., 2008; Zufferey *et al*., 2011). A hypothesis for this susceptibility has been proposed and linked to hydraulic vulnerability segmentation (Hochberg *et al*., 2016). This hypothesis predicts that the organs most likely to undergo embolism in a plant are those that are distal and expendable, instead of the main stem of the plant.

However, it has been indicated that small xylem vessels of the petiole could contribute to a cultivar's adaptation to water deficit (Hochberg *et al.,* 2014). 'Cabernet Sauvignon' is noted for small petiole xylem vessels in both well-watered and water deficit conditions therefore some cultivars are 'preadapted' for drought conditions (Hochberg *et al*., 2014). Figure 2D illustrates the main vein with bicollateral xylem vessels, varying in width in the cultivar, 'Cabernet Sauvignon'. A smaller vessel size acts as a strategy to prevent excessive water loss due to a reduction in hydraulic conductivity, thus possibly preventing embolism (Tyree and Dixon, 1986; Lovisolo *et al*., 2010; Davis *et al*., 1999).

A high petiole specific hydraulic conductivity tends to be correlated with a occurrence of larger diameter xylem vessels (Scholander *et al*., 1955; Esau, 1965; Lovisolo and Schubert, 1998), as also observed in the cultivars 'Grenache' (near isohydric) and 'Chardonnay' (anisohydric) (Shelden *et al*., 2017). In this case, 'Grenache' samples presented a higher petiole specific conductivity, due to the high frequency of larger xylem vessel diameters, suggesting that 'Grenache' is hydraulically adapted to supply a larger leaf surface area. A similar pattern was noted when studying the rachis xylem of 'Grenache' and 'Shiraz' (Scharwies and Tyerman, 2017). However, it should be taken into account that the dichotomy between iso and anisohydric cultivars should be used with care as genotypes may present different responses to imposed stresses and growing conditions, which will affect stomatal regulation (Schultz, 2003; Chaves *et al.,* 2010; Lovisolo *et al*., 2010; Rogiers *et al.,* 2012; Chaves *et al.,* 2016).

Petiole hydraulic conductivity and vine water status do not always correlate, as was the case with 'Grenache' cultivar (Shelden *et al*., 2017). However, the direct correlation of a decline in petiole hydraulic conductivity and an increase in water stress, as portrayed by the cultivar 'Chardonnay', could be as a result of a decline in the water conducting pathway permeability. The hydraulic properties and water management strategies of cultivars could be taken into account when considering a sustainable irrigation strategy, especially in dry and hot regions.

Grapevine petioles have demonstrated vulnerability to cavitation under heat stress conditions, leading to leaf senescence and stem preservation against further water stress (Keller, 2005). The distribution of xylem vessels is often different between cultivars; for instance, it was discovered that the petioles of 'Shiraz' had lower frequency of small vessels (<600 μm^2) and higher occurrence of larger vessels (>600 μm^2) (Hochberg *et al*., 2014). The same study scrutinises the lack of rigor in some previous studies and underlines the importance of consistency in trials. For instance, it was also pointed out that the cutting of xylem under tension, can trigger cavitation; this would of course, skew the results and not provide a realistic field scenario (Wheeler *et al*., 2013).

All facts considered, it seems that when under water deficit, the petiole xylem vessels of grapevine leaves are capable of embolization before the stem xylem vessels. Once embolism is induced in the petiole xylem, water loss is further limited and this helps maintaining the water functionality in the grapevine stem (Hochberg *et al.,* 2016).

Shoot and stem hydraulic conductivity

It is understood that water stress causes a decrease in size of grapevine vessels as well as xylem hydraulic conductivity (Lovisolo and Schubert, 1998; Lovisolo *et al*., 2002). Embolism following cavitation can lead to a decrease in hydraulic conductivity of the affected plant organ, thus

influencing leaf water status and limiting leaf gas exchange (Rood *et al.,* 2000; Davis *et al*., 2002; Brodribb and Cochard, 2009).

As outlined, grapevine stem hydraulic conductivity not only depends on water availability and environmental factors, but also on the xylem architecture (Choat *et al*., 2010). The way in which the xylem architecture adapts to abiotic and biotic factors will affect the hydraulic conductivity, maybe through cavitation, resulting in hydraulic failure and/or embolism or simply, reducing in size. It is also important to note the presence of other vessels, containing tyloses or gels, that function non-hydraulically (Pagay *et al.,* 2016). Furthermore, the presence of nodes is known to reduce conductivity and may help to explain the differences in theoretical and actual hydraulic conductivity measurements (Jacobsen *et al*., 2015).

According to Tyree and Sperry (1988), when under conditions of ever-present water stress, all plant species function near the tipping point of xylem failure.

Grapevine xylem hydraulic properties are often correlated with the region of origin of the cultivar but irrigation is often used as a way of obtaining high yields and quality, meaning that cultivar specific differences are often skewed and thus, unconsidered in studies (Pouzoulet *et al.,* 2020). Other sources claim that the hydroactive xylem area can vary along light gradients within the canopy (Oren *et al.,* 1986).

Although studies have attempted to make correlations between xylem vessel diameter and the probability of hydraulic failure (Hargrave *et al*., 1994), it is believed that there must be a compromise between xylem vulnerability and hydraulic efficiency (Cai and Tyree, 2010). However, a clear correlation between vulnerability and vessel diameter is not always present as observed amongst the cultivars 'Cabernet Sauvignon', 'Syrah' and 'Carménère' (Quintana-Pulido *et al*., 2018). On the contrary, this study emphasizes that other xylem traits such as the number, total area and structure of pits, could be equally important when evaluating grapevine xylem vulnerability to cavitation as also mentioned in various studies involving other plant species (Choat *et al*., 2004; Wheeler *et al*., 2005; Christman *et al*., 2009; Brodersen *et al*., 2013a; Knipfer *et al*., 2018).

CONCLUDING REMARKS

This review shows that a large degree of variability in morphoanatomy exists among *V. vinifera* cultivars. This makes it difficult to find direct correlations as there are many factors involved, which affect each trait differently. Furthermore, leaf morphoanatomy is clearly a complex topic, requiring specialised equipment and expertise in order to guarantee reliable results in scientific trials. Overall, given its importance, to the best of our knowledge, this area seems to be understudied.

However, this review shows that certain expressions of morphoanatomical traits may be advantageous in areas of increased abiotic stress. Cultivars with a comparatively low leaf area, high stomatal density but a lower stomatal dimension, as well as abundant intercellular spaces in the spongy layer of the mesophyll, may contribute to climate change resilience in hot and dry climates. The indumentum, a lesser known but important ampelographic descriptor, should be considered, as cultivars exhibiting few to no leaf hairs may be inappropriate in dry and hot grape growing regions. Grapevine xylem vessels are long and wide, which allow higher hydraulic conductivity, but this also makes them more prone to cavitation when subjected to water stress. Petiole xylem vessels are proven to be more susceptible to embolism than stem xylem vessels and can be linked to hydraulic vulnerability segmentation.

Despite the adaptations required at a cultivar level, climate change will bring new opportunities, with the emergence of vineyards in new regions and the possibility to plant cultivars from warmer regions in cooler areas. The more is known about the impact of climate change amongst cultivars and the vineyard ecosystem, the more the capacity to ensure the successful preservation of cultivars. Furthermore, genetic breeding programmes need to focus more on abiotic stress tolerance. Solutions for coping with abiotic stress are likely to be vineyard specific given the global diversity of cultivars, climate and topography. Additionally, it is fundamental to pay more attention to the relevance of morphoanatomical traits regarding their contribution to cultivars adaptability. This is going to be increasingly pertinent as water resources become scarcer.

As only on a few cultivars have been studied, future research should be expanded to other cultivars and terroirs. If grape growers were more aware of the features revealed by such studies, it would help them choose the best suited cultivar(s) for a specific site as well as adopt suitable management strategies.

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